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PECRASSIPINES A AND B, SECO-BISBENZYLISOQUINOLINE ALKALOIDS FROM *PHAEANTHUS CRASSIPETALUS*

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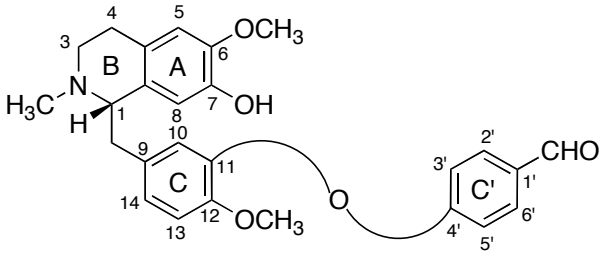
Abstract – Chemical investigation on the bark of *Phaeanthus crassipetalus* (Annonaceae) yielded two new *seco*-bisbenzylisoquinoline alkaloids, (+)-pecrassipine A (**1**) and (-)-pecrassipine B (**2**), together with seven known alkaloids. Their structures were elucidated by two-dimensional NMR techniques. Pecrassipines A and B exhibited a vasorelaxant activity on isolated rat aorta ring.

INTRODUCTION

Genus *Phaeanthus* was reported as a rich source of alkaloids.[1] There are 20 species distributed in South India, Lower Burma, Cambodia, Malay Peninsula to New Guinea, and the Philippines.[2] In Malaysia, only two species, *Phaeanthus crassipetalus* and *P. opthalmicus* are found.[2,3] Fasihudin *et al.* has reported the occurrence of oxoaporphine and bisbenzylisoquinolines from *Phaeanthus crassipetalus* collected from Sabah (East Malaysia), which has been used by the locals in Sabah to treat wounds and high blood pressure.[3]

Chemical investigation on extracts of the bark of *Phaeanthus crassipetalus* Becc. collected from Dungun Forest, Terengganu (West Malaysia) resulted in the isolation of two new *seco*-bisbenzylisoquinoline alkaloids, pecrassipines A (**1**) and B (**2**), which inhibited vasocontraction induced by norepinephrine (NE) on rat aorta, together with seven known alkaloids, doryphornine methyl ether,[4] thalifoline,[5] lanuginosine,[6]

seco-bisbenzylisoquinoline; (+)-vietnamine (**4**),[1b] and three bisbenzylisoquinolines; (-)-*O*-methyldauricine,[7] (-)-limacine,[8] and (+)-limacusine.[9] This paper describes the isolation and structural elucidation of **1** and **2** with a moderate vasorelaxant activity.



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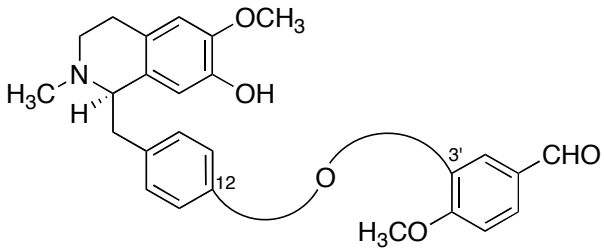

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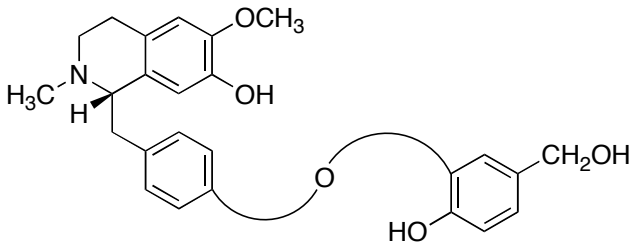

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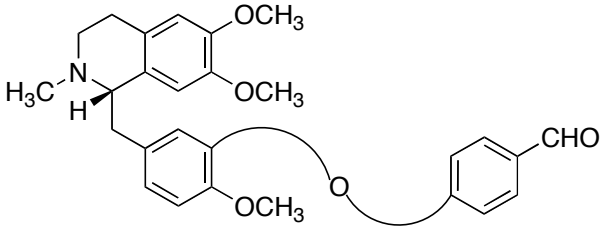

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RESULTS AND DISCUSSION

Pecrassipine A (**1**) was obtained as brownish solid, $[\alpha]_D^{26}$ +27 (*c* 0.4, MeOH). HRESIMS spectrum showed the $[M+H]^+$ at *m/z* 434.1943 corresponding to $C_{26}H_{28}NO_5$. EIMS of **1** showed a major fragment ion at *m/z* 192 (base peak) representing the upper part fragment of **1** (rings A and B). IR absorptions implied the presence of hydroxyl (3413 cm^{-1}) and conjugated carbonyl (1689 cm^{-1}) functionalities. UV spectrum showed an absorption maximum at 298 nm due to extend of conjugation.

Analysis of ^{13}C NMR data (Table 1) and the HMQC spectrum of **1** revealed the presence of nine sp^2 quaternary carbons, ten sp^2 methines, one sp^3 methine, three sp^3 methylenes, and three methyl groups. Among them, one sp^3 methylene (δ_C 47.5; δ_H 2.72 and 3.08) and methine (δ_C 64.3; δ_H 3.76), and a methyl (δ_C 41.8; δ_H 2.53) were ascribed to those bearing a nitrogen atom, while five sp^2 quaternary carbons (δ_C 142.3, 143.5, 145.5, 149.9, and 163.6), one sp^2 methine (δ_C 190.9), and two methyls (δ_C 55.7 and δ_C 55.9) were ascribed to those bearing an oxygen atom.

The ^1H NMR spectrum of **1** displayed an AA'BB' system of a *para*-disubstituted benzene moiety [δ 7.78 (2H, *d*, *J*=8.3 Hz, H-2', H-6') and 6.92 (2H, *d*, *J*=8.3 Hz, H-3', H-5')]. In addition, an ABX coupling system was observed at δ 6.72 (1H, *d*, *J*=2.0 Hz, H-10), 6.91 (1H, *d*, *J*=8.3 Hz, H-13), and 7.03 (1H, *dd*, *J*=8.3, *J*= 2.0 Hz, H-14). Two aromatic singlets were present at δ 6.49 and 6.22 assignable to H-5 and H-8 of ring A. The aldehyde proton at C-1' appeared downfield as a singlet at δ 9.87. Two methoxy

signals attached to C-6 and C-12 were observed at δ 3.80 and 3.75, respectively, while the *N*-methyl singlet appeared at δ 2.53. The presence of NOESY correlations between H-5 and the methoxy signal at δ 3.80, and between H-13 and that at δ 3.75 confirmed their positions at C-6 and C-12, respectively.

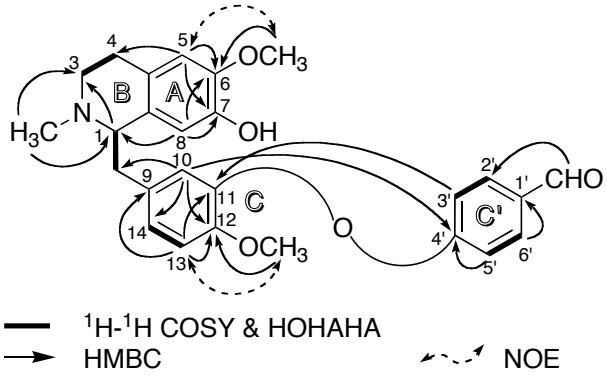

Figure 1. Selected 2D NMR correlations for pecrassipine A (**1**)

Figure 1 showed selected 2D NMR correlations for pecrassipine A (**1**). HMBC correlations for *N*-CH_3 of C-1 and C-3, and H-1 of C-3 gave rise to the connectivity among C-1, C-3, and *N*-CH_3 through a nitrogen. COSY and HMBC correlations for H-10 to C-α (δ_C 40.1) showed the presence of a 1-benzylisoquinoline ring. Connectivities between C-11 in ring C and C-4' in ring C' through an ether linkage were elucidated by HMBC cross-peaks for H-10 to C-4' and H-3' to C-11.[10] Thus, the gross structure of pecrassipine A was elucidated to be **1** with *seco*-bisbenzylisoquinoline skeleton with a benzaldehyde moiety through an ether linkage as shown in Fig. 1. The absolute configuration at C-1 was determined by comparing the CD spectrum with that of (+)-karakoramine (**3**) with an S configuration. The CD spectrum of **1** showed a positive Cotton effect at 217 nm indicated an *S* configuration which is in accordance with observation of **3**.[11]

Pecrassipine B (**2**) was isolated as a brownish solid with $[\alpha]_D^{26}$ -21° (*c* 0.8, MeOH). HRESIMS spectrum of **2** exhibited an $[M+H]^+$ at *m/z* 434.1953, which is consistent with the molecular formula of $C_{26}H_{27}NO_5$. The base peak, *m/z* 192, representing the isoquinoline moiety, was identical with that of **1**. IR spectrum showed absorptions at 3343 and 1687 cm^{-1} due to hydroxyl and conjugated carbonyl functionalities, respectively. UV spectrum showed an absorption band at 296 nm. The ^1H NMR spectrum of **2** is similar to those of **1** except for observation in three aromatic protons; H-2', H-5', and H-6' of the ring C' appeared as an ABX system. Additional methoxy group in ring C' was placed at C-4' by HMBC correlations for H-6' and 4'-OCH_3 to C-4'.

The assignments of all the carbons were established by HMQC, HMBC, and DEPT experiments (Table 1). The monoether lingkage of **2** (C-12–O–C-3') was also confirmed from the HMBC spectrum that showed the long range correlation between H-11 and C-3'. The CD Cotton curve showed negative at 233 nm, which was diagnostic of an *R* absolute configuration at C-1. The negative sign of the specific rotation, also confirmed this stereochemical assignment. In addition, we also report the absolute configuration of

vietnamine (**4**) since it was not determined previously. The CD spectrum of **4** showed a positive Cotton effect at 233 nm, thus denoting an *S* absolute configuration at C-1. Furthermore, vietnamine (**4**) gave a positive specific rotation of $[\alpha]_D^{26}$ +28° (*c* 1.4, MeOH).

Pecrassipines A (**1**) and B (**2**) were assayed for vasorelaxation effects on isolated rat aorta using a reported procedure.[12] Both pecrassipines A (**1**) and B (**2**) (10^{-4} M) showed relaxation responses against norepinephrine (NE, 3 x 10^{-7} M) induced contraction of rat aorta strips with endothelium after achieving a maximal response (**1**, 82%; **2**, 70%). Pecrassipine A (**1**) exhibited moderate vasorelaxant activity, although the activity was not so potent as compared to that of curine, bisbenzylisoquinoline alkaloid from *Chondrodendron platyphyllum*.[13] Pecrassipine B (**2**) showed slow vasorelaxant actions.

Table 1. ^{1}H [δ_H (J, Hz)] and ^{13}C NMR Data [δ_C] of pecrassipines A (**1**) and B (**2**) in $CDCl_3$

Position	δ_C	δ_H (*J* Hz) **1**	δ_C	δ_H (*J* Hz) **2**
1	64.3	3.76 *m*	64.7	3.89 *m*
3	47.5	2.72 *m*, 3.08 *m*	48.3	2.90-2.95 *m*
				3.26-3.85 *m*
4	25.4	2.53 *m*, 2.81 *m*	25.6	2.62 *m*, 2.90 *m*
4a	124.5	-	124.7	-
5	110.6	6.49 *s*	110.6	6.56 *s*
6	145.5	-	146.8	-
7	143.5	-	143.8	-
8	113.7	6.22 *s*	113.9	6.18 *s*
8a	129.7	-	129.8	-
9	130.7	-	130.5	-
α	40.1	2.70-2.81 *m*	40.4	2.90-2.95 *m*
		3.04-3.14 *m*		3.27-3.86 *m*
10	123.8	6.72 *d* (2.0)	131.2	7.05 *d* (8.3)
11	163.6	-	118.4	6.87 *d* (8.3)
12	149.9	-	146.0	-
13	112.8	6.91 *d* (8.3)	118.4	6.87 *d* (8.3)
14	127.3	7.03 *dd* (8.3, 2.0)	131.2	7.05 *d* (8.3)
1'	132.1	-	130.1	-
2'	131.8	7.78 *d* (8.3)	118.8	7.39 *d* (1.4)
3'	116.3	6.92 *d* (8.3)	155.2	-
4'	142.3	-	156.0	-
5'	116.3	6.92 *d* (8.3)	113.9	7.10 *d* (8.0)
6'	131.8	7.78 *d* (8.3)	127.6	7.62 *dd* (8.0, 1.4)
1'-CHO	190.9	9.87 *s*	190.6	9.80 *s*
6-OCH_3	55.9	3.80 *s*	56.2	3.85 *s*
4'-OCH_3	-	-	55.8	3.95 *s*
12-OCH_3	55.7	3.75 *s*	-	-
N-CH_3	41.8	2.53 *s*	40.7	2.55 *s*

EXPERIMENTAL

General Experimental Procedures. The physical data were recorded from the following instruments: UV spectra, Shimadzu UV-160A ultraviolet-visible spectrophotometer; CD spectra, Jasco J-820 spectropolarimeter; IR spectra, Perkin Elmer 1600 double-beam recording spectrometer; Optical rotations, JASCO P1010 with tungsten lamp; HRMS, Automass Multi Thermofinnigan spectrometer; 1D and 2D NMR, JEOL FT-NMR lambda 400 MHz. Chemical shifts were reported using residual $CDCl_3$ (δ_H 7.26 and δ_C 77.0) as internal standard. Standard pulse sequences were employed for the 2D NMR experiments.

Plant Material. The barks of *Phaeanthus crassipetalus* Becc. were collected at Dungun, Terengganu, Peninsular Malaysia in 1996. The botanical identification was made by Mr. Teo Leong Eng, Faculty of Science, University of Malaya. A voucher specimen (KL 4627) is deposited at the Herbarium of the Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia and also at the Herbarium of the Forest Research Institute, Kepong, Malaysia.

Extraction and Isolation. The dried, grounded bark of the plant (2.3 kg) was first defatted with hexane for 24 h. The residual plant material was dried up and left overnight after moistening with 10 % NH_4OH. They were then re-extracted with CH_2Cl_2 by Soxhlet extractor for 17 h. After filtration, the supernatant obtained was concentrated to 500 mL followed by acidic extraction with 5% HCl until a negative Mayer's test. The aqueous solution obtained was basified with NH_4OH to pH 11 and re-extracted with CH_2Cl_2. This was followed by washing with distilled H_2O, dried over anhydrous sodium sulphate, and evaporated to give a crude alkaloid fraction (18.5 g).

The crude alkaloids (6.0 g) were subjected to column chromatography over silica gel using CH_2Cl_2 gradually enriched with methanol to yield 190 fractions. Fractions 156-158 (105 mg) were subjected to silica gel column using CH_2Cl_2-MeOH (98:2) as eluent to afford pecrassipine A (**1**, 40.1 mg, 0.005% yield). Fractions 162-164 (23.8 mg) were subjected to CC on silica gel with solvent system CH_2Cl_2-MeOH (97:3) to afford pecrassipine B (**2**, 11.1 mg, 0.001% yield). From the other fractions, 4',5-diformyl-2-methoxydiphenyl ether, doryphornine methyl ether, lanuginosine, (+)-vietnamine (**4**), thalifoline, (-)-*O*-methyldauricine, limacine, and limacusine were isolated as known alkaloids by preparative TLC and CC on silica gel with CH_2Cl_2 – MeOH solvent system.

Pecrassipine A (1): brownish solid; $[\alpha]_D^{23}$ +27° (c 0.4, MeOH); UV (MeOH) λ_{max} 298 nm (ε 8200); IR (liquid film) ν_{max} 3413 (OH), 2913, 1689 (C=O), 1509 (aromatic ring), and 1227 cm^{-1}; CD mdeg (nm) +14 (233); HRESIMS *m/z* 434.1943 (M+H; calcd for $C_{26}H_{28}NO_5$, 434.1967). EIMS *m/z* 434 and 192; ^{1}H-NMR and ^{13}C-NMR see Table 1.

Pecrassipine B (2): brownish solid; $[\alpha]_D^{23}$ -21° (c 0.8, MeOH); UV λ_{max} (MeOH): 296 (ε 8100); IR (liquid film) ν_{max} 3343(OH), 1687 (C=O), 1597, and 1023 cm^{-1}; CD mdeg (nm) +71 (233); HRESI *m/z*

434.1953 (M+H; calcd for $C_{26}H_{28}NO_5$, 434.1967); EIMS *m/z* 434 and 192; ^{1}H-NMR and ^{13}C-NMR see Table 1.

Vasodilator Assay.[12] A male Wistar rat weighting 340 g was sacrificed by bleeding from carotid arteries under an anesthetization. A section of the thoracic aorta between the aortic arch and the diaphragm was removed and placed in oxygenated, modified Krebs-Henseleit solution (KHS: 118.0 mM NaCl, 4.7 mM KCl, 25.0 mM $NaHCO_3$, 1.8 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 1.2 mM $MgSO_4$, and 11.0 mM glucose). The aorta was cleaned of loosely adhering fat and connective tissue and cut into ring preparations 3 mm in length. The tissue was placed in a well-oxygenated (95% O_2, 5% CO_2) bath of 10 ml KHS solution at 37°C with one end connected to a tissue holder and the other to a force-displacement transducer (Nihon Kohden, TB-611T). The tissue was equilibrated for 60 min under a resting tension of 1.0 g. During this time the KHS in the tissue bath was replaced every 20 min.

After equilibration, each aortic ring was contracted by treatment with 3×10^{-7} M norepinephrine (NE). The presence of functional endothelial cells was confirmed by demonstrating relaxation to 10^{-5} M acetylcholine (Ach), and aortic ring in which 80% relaxation occurred, were regard as tissues with endothelium. When the NE-induced contraction reached plateau, each sample was added.

These animal experimental studies were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University and under the supervision of the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Science, Sports Culture, and Technology of Japan.

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